UNIVERSITY of GUELPH

INSTITUTE FOR COMPARATIVE CANCER INVESTIGATION

13th Annual ICCI

Cancer Research Symposium

Thursday May 27, 2021

HopIn Web Platform 9:00-3:00

Introductory Remarks

Welcome to the 13th annual Guelph ICCI Cancer Symposium! This meeting is an opportunity to bring together cancer researchers from across campus, as well as regional and national collaborators. Topics range from basic science through to clinical application. We are very grateful to the amazing group of speakers and poster presenters who will be sharing their findings with us today. Dr. Lisa Forrest is the 2021 Arthur Willis Distinguished speaker and will be giving the keynote address at 11:35.

In the past 14 years we have seen relationships and collaborations develop that were made possible by these interactions and we hope that this year's meeting will spark new collaborations and ideas.

This symposium is made possible by funding from the Arthur Willis Visiting Professorship in Canine Oncology and support from the OVC Dean's office.

Drs Geoff Wood and Michelle Oblak
Pathobiology and Clinical Studies, University of Guelph
ICCI Co-Directors

Administrative Support and Research Funding:

Thanks to Deirdre Stuart from the ICCI tumour bank for huge help organizing this symposium along with support from Scott Moccia from the OVC Office of the Dean, Zach Henderson from Conference Services for help with the virtual meeting platform, and Dr. Vicky Sabine for help with abstract reviews.

The research projects presented here and the trainees performing these studies were collectively supported by grants, scholarships and contracts from: Allard Research Chair Start-Up Fund; American Institute for Cancer Research; Brock Doctoral Scholarship; Canadian Cancer Society Research Institute; Cancer Research Society; CIHR; COVID-19 Rapid Research Fund; Ethel Rose Charney Scholarship in the Human/Animal Bond; Hass Avocado Board; Leukemia & Lymphoma Society of Canada; Leukemia Research Foundation; National Centre of Excellence in Biotherapeutics for Cancer Treatment (BioCanRx); NSERC; OICR; Ontario Research Fund; Ontario Veterinary College; OVC Graduate Scholarship; OVC Graduate Stipend Program; OVC Pet Trust Fund; Saskatchewan Health Research Foundation (SHRF); Smiling Blue Skies Cancer Fund; Stem Cell Network; Terry Fox Research Institute; The Canadian Hematology Society, and Vanier Canada Graduate Scholarship.

ICCI 13th Annual Cancer Research Symposium, Thursday May 27th, 2021

9:00-9:05 Welcome and Introductory Remarks

9:05-9:40 Guest Speaker

Standardized assessment, risk variables and survival in dogs with myelodysplastic syndrome and acute myeloid leukemia

Dr. Dorothee Bienzle; Department of Pathobiology, University of Guelph

9:40- 10:15 Short talks from abstracts

1. *Fatty acid oxidation is critical to AML mitochondrial metabolism.*
   Matthew Tcheng; Department of Food Sciences, University of Guelph
2. *Re-expression of the miR-200c/141 cluster in claudin-low mammary tumour cells alters cellular morphology, inhibits proliferation and invasion, and impairs mammary tumour initiation, growth and metastasis.*
   Kaitlyn Simpson; Department of Biomedical Sciences, University of Guelph
3. *The effects of proteasome inhibitors on canine B-cell lymphoma cell viability in vitro.*
   Nicholas Prevedel; Department of Biomedical Sciences, University of Guelph

10:15-10:25 ***Coffee Break and Poster Viewing***

10:25-11:00 Guest Speaker

Better die to be noticed: activating immunogenic cell death to reinstate tumor immunosurveillance.

Dr. Samuel Workenhe; Department of Pathobiology, University of Guelph

11:00-11:35 Short talks from abstracts

1. *Validation of explant cultures as a method for the identification of prognostic markers and molecular targets in canine osteosarcoma.*
   Anita Luu; Department of Biomedical Sciences, University of Guelph
2. *Investigating the hypoxia-induced alternative splicing event of eukaryotic ribosomal protein S24.*
   Jenna Kerry; Department of Molecular and Cellular Biology, University of Guelph
3. *Prognostic impact of tissue and serum urokinase plasminogen activator system in dogs with appendicular osteosarcoma.*
   Dr. Arata Matsuyama, Department of Clinical Studies, University of Guelph

11:35-12:40 Keynote Speaker

Cancer Imaging and Radiotherapy in Comparative Oncology

Dr. Lisa Forrest; Radiation Oncology, University of Wisconsin-Madison School of Veterinary Medicine

12:40-12:45 ***Closing Remarks***

12:45- 1:45 ***Lunch Break and Poster Viewing***

1:45-3:00 ***Interactive Poster Session***

KEYNOTE PRESENTATION

11:35 – 12:40 – Main Stage

Dr. Lisa Forrest, DVM, Diplomate ACVR (Radiology, Radiation Oncology)

Professor -in Radiology and Radiation Oncology at University of Wisconsin-Madison

Cancer Imaging and Radiotherapy in Comparative Oncology

Spontaneous cancers in companion (pet) animals have a long history of providing information regarding cancer biology and treatment strategies. Companion animal dogs have anatomic and physiologic similarities to humans, living in the same environment and being exposed to the same toxins. Specific tumor types in dogs that provide models for human cancer include osteosarcoma, nasal tumors, malignant melanoma, non-Hodgkin's lymphoma, soft tissue sarcomas and others. All these tumors occur spontaneously, and the course of disease is much shorter as compared to humans, which allows rapid data accrual. Medical equipment made for humans are accommodate canine patients, allowing use of state of the art imaging and radiotherapy machines.

Many advances in radiation oncology have occurred starting in the late 1980s. Intensity-modulated radiotherapy IMRT) and image-guided radiotherapy (IGRT) have been revolutionary for accurate treatment of tumors and sparing of tissue.

Development of helical tomotherapy with its highly precise delivery and verification system in the early 2000s began the development of conformal avoidance, meticulous and practical immobilization, adaptive radiotherapy and dose escalation. Dogs with nasal tumors provided the pre-clinical model to test helical tomotherapy and have been important in development of advances in dose painting using functional imaging, PET-CT, which is being incorporated into radiotherapy to personalize treatments.

Advances in radiotherapy are continuing with the goal of accurate tumor targeting and conformal avoidance of normal tissues with the goal of increasing survival with excellent quality of life.

Dr. Forrest received her DVM from the University of Pennsylvania School of Veterinary Medicine and subsequently completed a residency in Veterinary Radiology at North Carolina State University College of Veterinary Medicine. Following her residency, she joined the faculty of the University of Wisconsin-Madison School of Veterinary Medicine (UWMSVM). She is a Diplomate of the American College of Veterinary Radiology in Radiology and Radiation Oncology, and is currently a Professor of Radiology and Radiation Oncology at UWMSVM, where

she performs clinical duties in diagnostic imaging and radiation oncology and teaches veterinary students, house officers and referring veterinarians. Dr. Forrest has over 90 peer-reviewed publications and 25 book chapters. Her research involves clinical trials using radiotherapy in client owned pet dogs with spontaneous tumors. These spontaneous tumors in dogs have similar biological behavior as their counterparts in humans. The information obtained from these clinical trials helps advance and inform the design of future trials in humans. Dr. Forrest's collaborate work with medical physicist colleagues is advancing knowledge and utilization of molecular imaging in the form of positron emission tomography (PET) to design individual radiotherapy plans. She has broad experience in treating different tumor types in companion animals (pet dogs and cats). In addition, Dr. Forrest was project leader on a previous grant investigating the conformal avoidance capabilities of Helical Tomotherapy: CA88960-05 (Mehta PI) Improving Cancer Outcome with Adaptive Helical Tomotherapy: Animal Clinical Trial of Conformal Avoidance: Dog Nasopharynx. Dr. Forrest was also a Co-Investigator on an RO1 grant investigating PET imaging agents as surrogates of tumor proliferation and hypoxia, Dose Painting Based on Hypoxia and Proliferative Response: CA136927(Jerai PI). She is currently a collaborator with colleagues at both UW-Madison School of Veterinary Medicine and Carbone Cancer Center at UW School of Medicine and Public Health on a VA-Merit Grant investigating T-cell-immunotherapies in dogs with malignant melanoma.

Past ICCI Symposium Arthur Willis Distinguished Speakers

2019	David M. Vail	2014	Deborah Knapp
2018	Daniel Gustafson	2013	David Argyle
2017	William Eward	2012	Timothy Fan
2016	Jaime Modiano	2011	Cheryl London
2015	Nicola Mason	2010	Matthew Breen

GUEST SPEAKER:

9:05-9:40

Standardized assessment, risk variables and survival in dogs with myelodysplastic syndrome and acute myeloid leukemia

Dr. Dorothee Bienzle; Department of Pathobiology, University of Guelph

Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are heterogeneous hematopoietic neoplasms that are challenging to diagnose and prognosticate. Grading schemes that correlate with prognosis are needed. The objectives of this study were to estimate survival of dogs diagnosed with MDS or AML, to assess the effect of predictor variables on survival, and to identify survival predictors based on parameters adapted from the 2016 World Health Organization WHO guidelines. A search of the electronic laboratory database at the University of Guelph using the terms “dysplas” and “leukemia” yielded 70 cases that met all inclusion criteria for assessment. A novel grading scheme, based on the 2016 WHO guidelines, was devised, and applied to blood and bone marrow cytology and histopathology samples. Kaplan-Meier survival curves were estimated. Individual predictor variables were determined, and a best-fitting Cox regression model was derived. Forty-two dogs were diagnosed with MDS and 28 with AML. Median survival of dogs with MDS was 384 days (95% confidence interval [CI] 61-not achieved) and 6 days (95% CI 1-15) for dogs with AML. The percentage of blood blasts, WBC and platelet count, and body weight were individual predictor variables of survival (p ~~<~~≤0.05) regardless of diagnosis with hazard ratios of 1.06, 1.02, 0.998 and 1.26, respectively. Hence, within the constraints of a retrospective study, application of this grading scheme yielded prediction of distinct survival times for dogs with MDS and AML, and several clinically relevant survival predictor variables.

GUEST SPEAKER:

10:25-11:00

Better die to be noticed: activating immunogenic cell death to reinstate tumor immunosurveillance.

Dr. Samuel Workenhe**;** Department of Pathobiology, University of Guelph

Tumors activate programmed cell death after metabolic disturbances, genotoxic stress, and infectious insults. Depending on the nature of lethal stimuli, dying cancer cells release inflammatory secretomes, such as danger molecules and cytokines/chemokines, to potentiate multiple aspects of the anticancer immunity cycle. More than 20 cytotoxic anticancer therapies, including chemoradiotherapy, exert antitumor immunity by activating immunogenic cell death (ICD). As of March 29, 2021, there are 199 clinical trials combining ICD-inducing chemoradiotherapy with immunotherapies. Unfortunately, these combination therapies show dismal toxicities and long-term side effects. There is an enormous opportunity to curb toxicity and enhance therapeutic efficacy by developing rationally designed ICD-inducers. My talk will explore how basic knowledge of ICD can be harnessed to design potent immunotherapies.

Oral Presentation Abstracts

SHORT TALKS FROM SUBMITTED ABSTRACTS

9:40-10:15 Session

Fatty acid oxidation is critical to AML mitochondrial metabolism

Matthew Tcheng[1], Alessia Roma[1], Nawaz Ahmed[1], Richard W. Smith[2], Preethi Jayanth[1], Mark D. Minden[3], Aaron D. Schimmer[3], David Hess[4], Kristin Hope[5], Kevin A. Rea[6], Tariq A. Akhtar[6], Eric Bohrnsen[7], Angelo D'Alessandro[7], Al-Walid Mohsen[8], Jerry Vockley[8], and Paul A. Spagnuolo[1]*

1 Department of Food Science, University of Guelph; 2 University of Waterloo Mass Spectrometry Facility, Department of Chemistry; 3 Princess Margaret Cancer Center, Ontario Cancer Institute, Toronto ON; 4 Robarts Research Institute, University of Western Ontario, London, ON; 5 Stem Cell and Cancer Research Institute (SCC-RI), McMaster University, Hamilton, ON; 6 Department of Molecular and Cellular Biology, University of Guelph; 7 Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; 8 Department of Pediatrics and Center for Rare Disease Therapy, UPMC, Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA & Department of Human Genetics, School of Public Health, University of Pittsburgh, Pittsburgh, PA

Acute myeloid leukemia (AML) is a devastating hematological malignancy characterized by the uncontrolled proliferation and accumulation of leukemia cells that fail to differentiate into functional myeloid cells. Induction chemotherapy has remained unchanged for the past four decades and induces severe treatment-related co-morbidities, preventing extended dose escalation in the mature patient population and underscoring the need for novel chemotherapeutics that are better tolerated by the patient population. In contrast with normal blood cells, AML cells exhibit a dysregulated metabolic phenotype characterized by increased reliance on higher rates of fatty acid oxidation (FAO) for survival. Previous studies have focused on the inhibition of the cell-surface CD36 and mitochondrial surface CPT1 to eliminate AML. Profiling of publicly accessible gene expression databases of AML patients identified the very long chain acyl-CoA dehydrogenase (VLCAD) as a novel anti-AML target. VLCAD (encoded by the gene ACADVL), an enzyme involved in long chain mitochondrial FAO, was found to be overexpressed and critical to AML mitochondrial metabolism. Since clinical-grade VLCAD inhibitors do not currently exist, a respirometry-based screen identified 16-heptadecyne-1,2,4-triol (AYNE) as a specific VLCAD inhibitor. Loss of VLCAD activity either through genetic ablation or pharmacological inhibition suppressed mitochondrial respiration and fat contribution to the tricarboxylic acid cycle, resulting in increased AML cell death as well as decreased proliferation, clonogenic potential and engraftment. Quantification of ATP as well as assessment of FAO and glycolytic metabolism with stable isotope tracers were conducted in both primary AML cells and normal hematopoietic cells, which demonstrated divergent cell fates following VLCAD inhibition. In leukemia cells, FAO inhibition at VLCAD triggered increased pyruvate dehydrogenase activity insufficient to modify glycolysis, prevent ATP depletion or avert AML cell death. In contrast, hematopoietic cells were spared with no alterations in mitochondrial metabolism as well as ATP levels. In support of a therapeutic window, AYNE was well-tolerated in murine xenograft studies, a well-established pre-clinical model to assess *in vivo* efficacy of novel AML therapeutics. Together, these results demonstrate the role of VLCAD in AML mitochondrial metabolism and highlight a novel metabolic vulnerability in this devastating malignancy.

Funding: Ontario Research Fund, Leukemia & Lymphoma Society of Canada, Ontario Institute of Cancer Research, American Institute for Cancer Research, Leukemia Research Foundation, Stem Cell Network, The Canadian Hematology Society, NSERC, Hass Avocado Board

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Re-expression of the miR-200c/141 cluster in claudin-low mammary tumour cells alters cellular morphology, inhibits proliferation and invasion, and impairs mammary tumour initiation, growth and metastasis

K. Simpson, G. Conquer-van Heumen, K. Watson, R. Moorehead*.
Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph

Triple negative breast cancer (TNBC) is one of the most aggressive breast cancer subtypes with an observed increased rate of metastasis. It is uniquely unresponsive to targeted therapies, resulting in poor overall survival. The claudin-low TNBC subtype is composed primarily of mesenchymal and mesenchymal-stem like characteristics. Previously, our lab has demonstrated that re-expression of the of the microRNA family, miR-200s, restore the epithelial phenotype and suppress growth and metastasis in a murine claudin-low mammary tumour cell line. The miR-200 family consists of the following clusters: miR-200c/141 and miR-200a/ 200b/429. To examine whether our previous results translated in a human claudin-low breast cancer cell line, MDA-MB-231, we tested the hypothesis that over expression of miR-200s would inhibit proliferation/growth and migration/metastasis of human claudin-low breast cancer cells *in vitro* and *in vivo*. Re-expression of the miR-200c/141 cluster reverted MDA-MB-231 cells to a more epithelial morphology, significantly slowed proliferation, and significantly inhibited invasion *in vitro*. Additionally, our preliminary data indicates that re-expression of the miR-200c/141 cluster in MDA-MB-231 claudin-low cells delayed tumour initiation and reduced tumour growth following intramammary injections in an immunocompromised mouse model. Moreover, miRNA sequencing and mRNA sequencing shed light on the complexity of miR-200 function in claudin-low TNBC, revealing that re-expression of miR-200c/141 altered the expression of other microRNAs and genes regulated by SUZ12. Collectively, these results indicate that re-expression of the miR-200c/141 cluster in claudin-low mammary tumour cells promotes a transition to an epithelial-like cellular morphology, reduces proliferation and invasion, and impairs mammary tumour initiation, growth, and metastasis, all of which may potentially be mediated by SUZ-12 regulated genes and differentially expressed microRNAs.

Funding: CIHR and OVC Graduate Scholarship

The effects of proteasome inhibitors on canine B-cell lymphoma cell viability *in vitro*.

Nicholas Prevedel[1], Kayla Richard[1], Geoff Wood[2], Brenda Coomber*[1].
1 Department of Biomedical Science, Ontario Veterinary College, University of Guelph; 2 Department of Pathobiology, Ontario Veterinary College, University of Guelph

Cancer is common in the domestic dog and lymphoma is among the most prevalent types of neoplasia in this species. Canine lymphoma is treated with chemotherapy, and a common multi-drug combination is CHOP, an acronym for cyclophosphamide, hydroxydaunorubicin, vincristine [oncovin], and prednisone. Our laboratory previously showed using NextGen RNAseq that PSMB1, a component of the 20S core subunit of the proteasome, was expressed at higher levels

in lymphoma from dogs with poor response to CHOP when compared to dogs with good response. Proteasome inhibitors, such as Bortezomib and Ixazomib, are currently being employed clinically in combination with CHOP for the treatment of human hematological malignancies, including lymphoma. Co-treatment of human lymphoma cell lines with bortezomib and CHOP also showed a synergistic effect on cell survival. We therefore hypothesize that proteasome inhibitors will be effective for the treatment of canine lymphoma and will be likewise synergistic to the CHOP compounds. The aim of this present study is to determine the effect of proteasome inhibitors on CLBL-1 cell viability, and to investigate its sensitizing effects to CHOP chemotherapy agents. CLBL-1 is a well-characterized canine B-cell lymphoma cell line, similar to human Diffuse Large B-Cell Lymphoma. We confirmed through western blotting that CLBL-1 cells express a detectable level of the PSMB1 protein. The effects of proteasome inhibitors and CHOP on CLBL-1 viability were determined using an Alamar Blue assay. After 48 hr exposure, the IC50 of Bortezomib was 16.05 nM and of Ixazomib was 63.39 nM. The IC50 after 48h hr exposure to the CHOP compounds cyclophosphamide, hydroxydaunorubicin, and vincristine were 1.623 uM, 36.4 nM, and 0.113 nM respectively. We are currently investigating CLBL-1 responses to CHOP reagents in combination with proteasome inhibition, as well as the effects of PSMB1 overexpression on CLBL-1 response to CHOP. The results of this study may have clinical utility, as proteasome inhibition and targeted PSMB1 inhibition could potentially improve CHOP responsiveness and decrease chemotherapeutic-related toxicity for canine lymphoma patients.

Funding: OVC Pet Trust

11:00-11:35 Session

Validation of explant cultures as a method for the identification of prognostic markers and molecular targets in canine osteosarcoma

A. Luu[1], M. Cadieux[1], M. Wong[1], R. Macdonald[1], R. Jones[1], G. Wood[2], A. Viloria-Petit[1]*;
1 Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph; 2 Department of Pathobiology, Ontario Veterinary College, University of Guelph

Osteosarcoma (OSA) is the most common bone tumour in canines and humans. In canine OSA, there is an urgent need for novel modalities effective at treating metastatic disease and biomarkers that could aid in clinical decision-making. Extracellular vesicles (EVs) represent a family of membrane-bound vesicles that contain various biomolecules such as DNA, mRNA, and proteins. The content of EVs has been demonstrated to be distinct amongst cancer types and reflect the cell of origin. Thus, profiling EV cargo could aid in our understanding of OSA biology and find novel therapeutic targets and biomarkers. Upon owner consent, tumour and normal bone samples were obtained after limb amputation. Samples were mechanically fragmented and incubated in culture media under standard conditions for 24 hours. Vesicles were isolated using size exclusion chromatography and subjected to characterization via immunoblotting, transmission electron microscopy and particle tracking analysis. To profile the protein content in isolated vesicle preparations, 8 tumour and 4 normal bone vesicle samples were subjected to high performance liquid chromatography-tandem mass spectrometry (HPLC MS/MS). Twenty-nine proteins were distinct and significantly upregulated in the OSA vesicle samples compared normal bone vesicle samples. A majority (45%) of these proteins were associated with protein metabolism. One molecule of particular interest was proteasome 26S subunit, non-ATPase 14 (PSMD14) which

showed clinical significance in publicly available datasets. Inhibition of PSMD14 *in vitro* using the compound capzimin demonstrated a decrease in pro-tumourigenic traits in metastatic-derived canine OSA cell lines. In this preliminary study, we have successfully created a pipeline to isolate and characterize EV content from canine OSA patient explants. This unique pipeline has the ability to increase our understanding of OSA and find new molecular targets for both human and canine OSA patients.

Funding: OVC Pet Trust

Investigating the hypoxia-induced alternative splicing event of eukaryotic ribosomal protein S24

J. Kerry, J. Uniacke*.
Department of Molecular and Cellular Biology, University of Guelph

In response to hypoxia (low oxygen), eukaryotic cells have developed mechanisms that control gene expression in order to survive. Given that hypoxia is a characteristic of the tumour microenvironment, hypoxic stress response mechanisms provide tumour cells with survival benefits, thereby increasing oncogenic potential. One mechanism in particular that has recently been referred to as the 11th hallmark of cancer is hypoxia-induced alternative splicing. Research has shown that hypoxia changes expression of splicing regulators, which, as a result, changes alternative splicing patterns of cancer-associated genes and promotes metastatic disease. Of particular interest is recent published data that suggests hypoxia also influences alternative splicing of ribosomal protein mRNAs, and more specifically, found a splicing event in ribosomal protein S24 (RPS24) that is greatly induced in spheroids (*in vitro* aggregates of tumour cells). Here, we show data that suggests that the hypoxia-induced alternative splicing event in RPS24 is, in part, due to the hypoxia inducible factor (HIF) response mechanism. We quantified the RPS24 long and short splice variants via quantitative polymerase chain reaction (qPCR) in the presence or absence of HIF1-α and HIF2-α. To knockdown the HIFs we used, HIF1-α inhibitor echinomycin and HIF2-α inhibitor TC-S 7009. To induce the HIFs we used, dimethyloxalylglycine (DMOG), as well as transfected stable HIF1-α and HIF2-α plasmids. Our data suggests that when HIF1-α and HIF2-α are present, as in hypoxia, there is a reduction in the RPS24 short variant. This is consistent with the literature in that the reduction of the RPS24 short variant aids in the increase of the RPS24 long:short variant ratio that has previously been shown in hypoxia/spheroids. Our data suggests that alternative splicing of ribosomal proteins may be part of the HIF response mechanism to increase cell survival during hypoxic stress.

Funding: NSERC.

Prognostic impact of tissue and serum urokinase plasminogen activator system in dogs with appendicular osteosarcoma.

Arata Matsuyama[1], Geoffrey A. Wood[2], Rachael Speare[2], Courtney R. Schott[2], Anthony J. Mutsaers[1,3].

1 Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph; 2 Department of Pathobiology, Ontario Veterinary College, University of Guelph; 3 Department of Clinical Studies, Ontario Veterinary College, University of Guelph

Urokinase plasminogen activator (uPA) and its receptor uPAR promote cancer invasion and metastasis and are emerging therapeutic targets in both human and canine malignancies. While their clinical significance is well-characterized in multiple human malignancies, studies investigating their roles in osteosarcoma are lacking. The objectives of this study were to characterize serum and tissue uPA/uPAR expression in dogs with osteosarcoma and assess their prognostic significance. Serum samples and a tissue microarray of canine appendicular osteosarcoma were analyzed for uPA and uPAR expression by ELISA (n=49) and immunohistochemistry (n=55), respectively. Serum uPA activity was also measured by chromogenic assay (n=25). Survival analysis was performed by Kaplan-Meier survival analysis, Log rank test, and Cox regression analysis. Serum uPA level was significantly higher in dogs with osteosarcoma (median 1905.4 pg/ml, 95% CI 1767.2-2043.6 pg/ml) than clinically healthy control dogs (median 1439.5 pg/ml, 95% CI 1071.8-1807.3 pg/ml, $p = 0.008$). The majority of canine osteosarcoma tissues expressed uPA (80.7%) or uPAR (80.7%), with 74.4% dual-positivity, indicating autocrine/paracrine activation of the pathway. Survival analysis revealed shorter progression free and overall survival in dogs with high serum uPA level both in a discovery cohort (n=29) and pooled samples (n=49), while serum uPAR and tissue uPA/uPAR levels were not prognostic. With Cox multivariate analysis, high serum uPA quantity and activity were both associated with shorter progression free ($p = 0.003$ and $p = 0.006$) and overall survival ($p = 0.008$ and $p = 0.026$), independent of serum ALP, tumor location, and peripheral lymphocyte/monocyte counts. Overall, these results indicate high utilization of the uPA pathway and association with disease progression in canine osteosarcoma. Further study involving prospective evaluation to confirm prognostic significance is warranted, and the high prevalence of tissue uPA and uPAR expression suggests that the uPA system is a potential therapeutic target in canine osteosarcoma.

Funding: OVC Pet Trust

INTERACTIVE POSTER SESSION 1:45-3:00
BOOTHS AREA

Posters will be displayed all day; authors please attend your posters as possible from 1:45-3:00. There will be no judging for the poster presentations this year.

POSTER ABSTRACTS

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1) Investigating the DEAD-box protein DDX28's regulation of HIF-2α and eIF4E2-directed translation initiation and its role in cancer in hypoxia

Olivia Bebenek and James Uniacke
Department of Molecular and Cellular Biology, University of Guelph.

Hypoxia (low oxygen) is a component of the tumour microenvironment, leading to increased metastasis, poorer patient prognosis and resistance to treatments like chemotherapy and radiotherapy. The Hypoxia Inducible Factors (HIFs) are hypoxia-stabilized heterodimeric transcription factors that regulate the expression of hypoxia-response genes that are necessary for survival. The lesser-studied α subunit, HIF-2α, is not only involved in transcriptional regulation, but also in hypoxic cap-dependent translation, as it is part of the hypoxic translation initiation complex that is led by the cap-binding protein eukaryotic initiation factor 4E2 (eIF4E2). It was recently suggested that the DEAD-box RNA helicase DDX28 may act as a tumour-suppressor protein by inhibiting eIF4E2-directed translation initiation through its interaction with HIF-2α. However, it is currently unknown which of DDX28's motif(s) are necessary for this regulation of HIF-2α and in which cellular compartment this interaction occurs. We hypothesized that DDX28 interacts with HIF-2α in the nucleus to inhibit it from participating in eIF4E2-directed translation initiation in the cytoplasm, as DDX28 is known to localize to the nucleus and nucleolus, but no function has been investigated. Additionally, because DDX28's primary function is in mitoribosome biogenesis, we proposed that DDX28 may localize to the nucleolus to regulate ribosome biogenesis. The transcript levels of several genes involved in ribosome biogenesis were examined in cells depleted of DDX28 in normoxia (normal oxygen levels) and hypoxia. Also, six EGFP-tagged DDX28 expression plasmids mutated at different motifs and localization signals known to cause localization changes were created, which will be transiently transfected into a U-87 MG human glioblastoma DDX28 "knockdown" cell line to 'rescue' previously described phenotypes. DDX28's normoxic and hypoxic localization was also investigated through subcellular fractionation. Finally, *DDX28* mRNA levels were surveyed in various cancer cells lines and compared to a non-cancerous cell line in normoxia and hypoxia. By further elucidating DDX28's influence on cancer progression, this research will not only aid in the overall understanding of hypoxic cell processes, but could lead to the development of novel cancer therapeutics.

Funding: NSERC

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2) Does oral apigenin have real potential for a therapeutic effect in the context of human gastrointestinal and other cancers?

Jonathan Blay[1,2]*, Eva F. Adem[1].
1 School of Pharmacy, University of Waterloo, Waterloo, ON, Canada, 2 Department of Pathology, Dalhousie University, Halifax, NS, Canada

Apigenin (4′, 5, 7-trihydroxyflavone) is a plant flavone that has been found to have various actions against cancer cells. We evaluated available evidence to determine whether it is feasible for apigenin to have such effects in human patients. Apigenin taken orally is systemically absorbed and recirculated by enterohepatic and local intestinal pathways. Its bioavailability is in the region of 30%. Once absorbed from the oral route it reaches maximal circulating concentration (Cmax) after a time (Tmax) of 0.5–2.5h, with an elimination half-life (T1/2) averaging 2.52 ± 0.56h.
Using a circulating concentration for efficacy of 1–5μmol/L as the target, we evaluated data from both human and rodent pharmacokinetic studies to determine if a therapeutic concentration would be feasible. We find that oral intake of dietary materials would require heroic ingestion amounts and is not feasible. However, use of supplements of semi-purified apigenin in capsule form could reach target blood levels using amounts that are within the range currently acceptable for other supplements and medications. Modified formulations or parenteral injection are suitable but may not be necessary. Further work with direct studies of pharmacokinetics and clinical outcomes are necessary to fully evaluate whether apigenin will contribute to a useful clinical strategy, but given emerging evidence that it may interact beneficially with chemotherapeutic drugs, this is worthy of emphasis. In addition, more effective access to intestinal tissues from the oral route raises the possibility that apigenin may be of particular relevance to gastrointestinal disorders including colorectal cancer.

Funding Sources: N/A

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3) Utilizing comparative oncology approach for the development of radioimmunotherapy for osteosarcoma

Jaline Broqueza[1], Chandra Bose Prabaharan[2], Samitha Andrahennadi[2], Kevin J.H. Allen[1], Ryan Dickinson[3], Valerie MacDonald-Dickinson[4], Ekaterina Dadachova[1]* and Maruti Uppalapati[2]*.
1 College of Pharmacy and Nutrition, University of Saskatchewan; 2 Department of Pathology and Laboratory Medicine, College of Medicine, University of Saskatchewan; 3 Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan; 4 Department of Small Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan.

Osteosarcoma (OS) is a type of bone cancer and is the fifth most common primary malignant bone tumor among adolescents and young adults. In over 30 years, there has been no significant progress in finding a new form of treatment for OS. Unfortunately, OS is also one of the most widespread cancers in companion dogs, particularly larger breeds. This project aims to develop a novel, effective and safe method of treating OS using a comparative oncology approach based on radioimmunotherapy (RIT). Recently, a protein called insulin-like growth factor 2 receptor (IGF2R) was found to be consistently overexpressed in different OS cancer cell lines and in human and canine patient samples, making it a promising therapeutic target for RIT. For this project, we developed IGF2R-specific antibodies by using phage-display, a technique in which bacteriophages are used to select for high affinity reagents. The IGF2R-specific antibodies developed will be used

as a delivery agent that will deliver cytotoxic radiation to the OS cells which overexpresses IGF2R thereby killing these cancer tumor cells. In essence, our research aims to develop a new and safe form of treatment for OS based on RIT and utilizing comparative oncology approach that will lead to new strategies to treat not only human OS but also OS of our non-human family members as well.

Funding: Canadian Institutes for Health Research (CIHR)

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4) Following administration of dendritic cell-based vaccines there is an increase in type 2 innate lymphoid cells in the local draining lymph node and spleen

Lily Chan, Sarah K. Wootton, Byram W. Bridle* and Khalil Karimi*.
Department of Pathobiology, Ontario Veterinary College, University of Guelph.

Innate lymphoid cells (ILCs) are separated into three main groups, type 1 (ILC1), type 2 (ILC2), and type 3 (ILC3), based on transcription factor expression, cytokine profiles, and specific phenotypic markers. ILC1, ILC2, and ILC3 mirror T helper (Th)-1, Th2, and Th17 responses, respectively, owing to their similar cytokine profiles, activities, and immune functions. ILC2s have been observed to have both pro-tumorigenic and anti-tumourigenic effects which appears to be dependent on the specific immunological context and the external stimuli they receive, indicating that their environment and surrounding circumstances will dictate if they have beneficial or adverse effects for an individual. Thus, there is potential to manipulate the local environment to induce desired phenotypes and responses of ILC2s, suggesting a possible role for ILC2s in immunotherapy. Dendritic cell (DC)-based vaccines are emerging as valuable cancer immunotherapeutic tools. DCs are potent antigen-presenting cells and act as the interface between innate and adaptive immunity. DC-based cancer vaccines aim to modulate the immune system to promote anti-tumour responses. Using murine models, we investigated the changes in ILC2 cellularity following DC-based vaccination. We observed a 6.8-fold increase in the number of ILC2s in the local draining lymph node and a 2.7-fold increase in the number of ILC2 producing IL-13 and IL-5 in the spleen following administration of a DC-based vaccine. We aim to investigate the crosstalk between DC-based vaccines and ILC2s to evaluate if their interactions can be modulated to improve anti-tumour responses.

Funding: Terry Fox Research Institute, OVC Pet Trust, CIHR, National Centre of Excellence in Biotherapeutics for Cancer Treatment, Canadian Cancer Society, NSERC, OVC.

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5) Impact of the miR-200 family on H3K27me3 levels and tumour microenvironment communication within Triple Negative Breast Cancer

G. Conquer-van Heumen, K. Simpson, K. Watson, R. Moorehead*
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Triple Negative Breast Cancer has the worst prognosis out of the main breast cancer subtypes, and currently has no approved specific treatments. The miR-200 family has previously been characterized as inhibiting the initiation, progression, and metastasis of Triple Negative Breast Cancer. Previous work in our lab demonstrated a capacity to induce dormancy, and mediate changes to the epigenetic marker H3K27me3. This study used both TNBC cell lines and tumours

which overexpressed the miR-200 family to investigate both immune-mediated and angiogenic-mediated mechanisms of tumour dormancy, along with the relationship between the miR-200 family and H3K27me3. Results indicated a significant positive relationship between the miR-200 family expression and H3K27me3 levels, implying that epigenetic modifications are a potential pathway for the exertion of the impacts brought on by miR-200 overexpression. Additionally, analysis of mechanisms of dormancy implicated both immune-mediated, and angiogenic-mediated dormancy as potential pathways for the miR-200 mediated dormancy effect demonstrated *in vivo*.

Funding: CIHR, OVC

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6) Evaluation of expression and function of the EphA2 receptor tyrosine kinase in canine and human osteosarcoma

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Osteosarcoma is a highly metastatic and lethal bone cancer in canines and humans. Although treatment is aggressive and consists of amputation and chemotherapy, the average duration of survival is one year post-treatment for dogs and only 60% of human patients survive longer than 5 years post-treatment. This study aims to better understand the pathophysiology of osteosarcoma with a focus on the role of the EphA2 receptor tyrosine kinase (RTK). EphA2 is one of nine members of the EphA RTK family and in the search for new targeted cancer therapies, EphA receptors are emerging as promising regulators of tumor development, invasiveness and drug resistance. However, the expression and potential roles of the EphA receptors in canine and human osteosarcoma have not been investigated. Our research has revealed the increased expression of EphA2 and EphA4 receptors in canine and human osteosarcoma cells lines using real-time PCR and Western blotting techniques. To evaluate the functional relevance of overexpressed EphA2 in osteosarcoma cells, we successfully silenced the expression of EphA2 in osteosarcoma cells using a specific shRNA. Silencing of EphA2 resulted in a reduced proliferation of osteosarcoma cells in culture when compared with non-silenced control cells. Silencing of EphA2 also reduced tumor growth rate in comparison to nonsilenced control cells in our mouse xenograft model. These data suggest that increased EphA2 function is a major driver of the malignant behaviour of dog and human osteosarcoma. In the future, I will assess the effect upregulated EphA2 receptor expression has on other aspects of osteosarcoma cellular function such as cell migration and drug sensitivity. We will also collaborate with Biomirex Inc. to develop synthetic antibodies to target EphA2 in human and dog osteosarcoma.

Funding: Allard Research Chair Start-up Fund and Saskatchewan Health Research Foundation (SHRF)

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7) Assessing the prognostic value of neutrophils in canine osteosarcoma

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Neutrophils play a vital role in maintaining the health of an organism, but the role they play in cancer is unclear. There is accumulating clinical evidence that elevated circulating neutrophil counts correlate with a poor prognosis in various cancers; however, little is known about the role of neutrophils in osteosarcoma (OSA), and their true prognostic value remains to be determined. The purpose of this retrospective study was to investigate whether neutrophils have prognostic significance in canine osteosarcoma (cOSA). In order to investigate this, cOSA patients treated with the standard of care (SOC) (amputation followed by chemotherapy) at the Ontario Veterinary College (OVC) over a 15-year period from 2006-2021 were evaluated for eligibility. A total of 84 cOSA patients were found to be eligible for this study. Using Kaplan-Meier analysis, we found that patients with a segmented neutrophil count higher than 7.9 x109/L before they underwent surgery had a significantly shorter time to metastasis and overall survival time. This association indicates that segmented neutrophils may have prognostic significance in cOSA patients, and that a pre amputation segmented neutrophil count higher than 7.9 x109/L may help predict a poor outcome in patients receiving the current SOC. OSA treatment has seen little improvement over the last 40 years. With our findings, segmented neutrophil counts show promise to help meet the significant unmet need for a prognostic biomarker to aid in owner and clinician decision making.

Funding: NSERC, OVC Pet Trust, OVC Stipend Scholarship

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8) Determining the effect of intrabody-mediated TERT suppression on cisplatin sensitivity and cellular behavior of human epithelial ovarian cancer cells

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Introduction: Platinum-based therapies are often used to treat epithelial ovarian cancer (EOC), but following initial treatment response, most patients experience therapy resistance and disease recurrence. TERT is the catalytic subunit of telomerase. Its main function is telomere maintenance, but it increases proliferation and reduces apoptosis via its telomere-independent functions. Recently, TERT has been associated with chemoresistance; four-fold higher expression was found in resistant compared to chemosensitive EOC cells, and nuclear to mitochondrial translocation was associated with chemoprotection. The following study uses an anti-hTERT intrabody to bind, suppress, and sequester TERT in EOC. We hypothesize this will reduce proliferation, increase apoptosis, and increase cisplatin sensitivity. Methods: A constitutive vector expressing an anti-TERT intrabody was constructed. Intrabody expression and nuclear localization were evaluated in normal ovarian surface epithelial (NOSE), and human ovarian cancer (OVCAR-3, and CAOV-3) cells. A dual-luciferase assay using a TERT promoter-containing firefly luciferase was designed to assess TERT promoter activity, and Southern blotting analysis was used to assess telomere degradation. Results: Plasmid construction was confirmed via sequencing. Intrabody expression was confirmed via western blotting and immunofluorescence displaying significant co-localization of nuclear and intrabody signals. CAOV-3 exhibited 72-fold greater hTERT promoter activity than NOSE cells suggesting selective activity and southern blotting analysis showed stable telomere lengths between treated and control cells for all lines. Conclusions: Successful intrabody expression and nuclear localization of intrabody was confirmed. The effects of intrabody binding on EOC behavior and chemosensitivity will be studied. This research will help elucidate

chemoresistance in EOC and determine the effects of anti-hTERT intrabody expression on EOC cells.

Funding: CIHR, CRS

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9) Investigating the effect of low anatomical temperatures on rhabdoviruses

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Viral vectors can be used to generate robust immunity against infectious diseases and cancers. While studied almost exclusively at 37°C, viruses are subject to a range of temperatures *in vivo* depending on their biodistribution. For example, viruses administered intranasally or into superficial tumors are tasked with replicating at temperatures as low as 31-34°C and 33°C, respectively. Despite the potential implications, the effect of temperatures ~~<~~≤37°C on the performance of viral vectors is unknown. We investigated this effect on the rhabdoviruses vesicular stomatitis virus (VSV) and Maraba virus (MG1). We utilized a VSV encoding a transgene for the truncated severe acute respiratory syndromecoronavirus-2 spike protein (VSV-SΔ19) and MG1 expressing a reporter transgene encoding enhanced green fluorescent protein (MG1-eGFP). Using a metabolic resazurin assay, the oncolytic ability of VSV-SΔ19 and MG1-eGFP was characterized in Vero, HeLa, and B16F10 cells at 28°C, 31°C, 34°C, and 37°C. VSV-SΔ19 and MG1-eGFP-mediated oncolysis was significantly hindered at 31°C and 28°C. This could result in a suboptimal immune response to these viral vectors and therefore the virotherapies will not be as effective. The effect of decreased temperatures on VSV-SΔ19 and MG1-eGFP replicative ability is under investigation using an *in vitro* growth curve assay at 28°C, 31°C, 34°C, and 37°C. As a potential mitigation strategy, cold-adaptation of these viruses is being attempted by serially passaging them at decreasing temperatures. Once replicating efficiently at 31°C the oncolytic and transgene-expressing abilities of cold-adapted VSV-SΔ19 and MG1-eGFP will be compared to the parental viruses at 31°C and 37°C. We hypothesize that cold-adapting VSV-SΔ19 and MG1-eGFP will increase their oncolytic ability and vaccination potential at low temperatures and outperform their parental counterparts *in vitro* and *in vivo*.

Funding: COVID-19 Rapid Research Fund (Ontario Ministry of Colleges and Universities) and an Innovation Grant (jointly funded by Canadian Institutes of Health Research - Institute of Cancer Research and Canadian Cancer Society).

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10) Differential susceptibility of viral vectors to elevated temperatures

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Viral vectors are powerful and versatile tools used to generate robust immune responses against infectious diseases and cancers. This class of therapeutics is generally well-tolerated in both animals and humans, often causing only mild side-effects such as fever. While fever plays a protective role during natural infection by enhancing immune activity and inhibiting pathogen

replication, it is unclear what effect it may have on the efficacy of viral vector platforms. We recently discovered that the oncolytic ability, transgene expression, and replication of the rhabdoviruses, vesicular stomatitis virus (VSV) and Maraba virus (MG1), were severely compromised at temperatures >37°C. As a mitigation strategy, we developed heat-adapted variants of VSV and MG1 that retained oncolytic and vaccine functions at temperatures up to 40°C. Expanding our investigation, we evaluated the effect of increased temperature on two additional prominent viral vectors, avian orthoavulavirus-1 (AOaV-1) and Orf virus (OrfV). Utilizing a resazurin dye-based metabolic assay, the oncolytic ability of OrfV and lentogenic versus mesogenic AOaV-1 was characterized in numerous murine and human cancer cell lines at 37, 38.5, and 40°C. In contrast to our previous findings with VSV and MG1 heat sensitivity, the oncolytic ability of lentogenic AOaV-1 and mesogenic AOaV-1 were minimally disrupted or unaffected at increased temperatures in a cell line-dependent manner. Interestingly, the oncolytic ability of OrfV was consistently enhanced at increased temperatures. The effect of temperature on *in vivo* efficacy of AOaV-1 and OrfV is currently under investigation in two murine tumour models of simulated fever. These results suggest that viral vectors may be differentially affected by temperature increases associated with fevers and that therapeutic efficacy may be enhanced through heat adaptation or use of heat-insensitive or heat-enhanced viruses. We recommend early screening of viral vectors for potential sensitivity to body temperatures >37°C so consideration can be given to temperature-related mitigation strategies.

Funding: Canadian Cancer Society Research Institute, Canadian Institutes of Health Research, and Natural Sciences and Engineering Research Council of Canada

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11) MicroRNA expression in serum of canine hemangiosarcoma and lymphoma patients

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MicroRNAs (miRNAs) are small, non-coding RNA molecules of approximately 22 nucleotides that are differentially expressed in bodily fluids in healthy and diseased states. Both hemangiosarcoma and multicentric lymphoma are highly malignant cancers in dogs, and many patients can benefit from early detection of disease to allow intervention before the cancer spreads from the site of origin. Our laboratory has previously shown that miRNAs are dysregulated in plasma of dogs with osteosarcoma and lymphoma at the time of diagnosis, and many of these miRNAs correlate with clinical outcome. However, there is minimal research on the miRNA profile of dogs with hemangiosarcoma or in serum of dogs for either of these cancers. The goal of this study is to characterize the miRNA expression profiles of serum in canine hemangiosarcoma and T and B cell multicentric lymphoma patients that presented to the OVC Health Sciences Center. These miRNA profiles will then be investigated in serum collected prior to diagnosis from samples we acquired from the Morris Animal Foundation's Golden Retriever Lifetime Study. Serum from age and sex-matched dogs with no malignant neoplasia, dogs diagnosed with hemangiosarcoma, and dogs diagnosed with B or T cell multicentric lymphoma were obtained from the ICCI Companion Animal Tumour Sample Bank. For initial profiling, samples were selected to be included in 2-3 pooled samples per group, with each pool consisting of 5 individual patients. After pooling, each pooled sample underwent miRNA isolation and RT-qPCR using QIAGEN miScript Canine miRNome PCR arrays. Fold expression changes were calculated using $2^{-\Delta\Delta Ct}$ and were considered differentially expressed if the fold expression change was >2. In

hemangiosarcoma dogs, 37 miRNAs were upregulated and 28 were downregulated compared to control dogs. In B and T cell lymphoma dogs, 37 and 29 miRNAs were upregulated, and 64 and 26 were downregulated, respectively, compared to control dogs. Multiple miRNAs were also differentially expressed between B and T cell lymphoma dogs. This data suggests that multiple miRNAs are differentially expressed in serum of dogs with hemangiosarcoma and B and T cell multicentric lymphoma compared to healthy dogs. As well, serum miRNAs may be able to differentiate between B and T cell lymphoma. These results will be used to select miRNAs for individual profiling of patient serum to determine potential early diagnostic and prognostic miRNAs.

Funding: OVC Pet Trust

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12) All about the gold: A novel approach to the treatment of localized prostate tumors in dogs.

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Prostate carcinoma (PC) in dogs is relatively rare, but this disease is challenging to diagnose and effectively treat. Like humans, dogs are the only other large mammal that naturally develops PC likely due to the shared morphology and functionality of the prostate gland. Current treatments in veterinary oncology for PC include surgical excision or stenting, intravenous chemotherapy and/or radiation therapy. Currently, there is no universally accepted standard of care treatment. Even with combined therapeutic approaches, prognosis of dogs with PC is poor. These tumors are in a difficult location and have a high rate of local invasion and widespread metastasis. Palliative measures, rather than curative, are most often employed for the treatment of dogs with PC. Gold nanoparticles (AuNP) have potential in biomedical applications, including cancer treatments. Using a "green" synthesis method, plant extracts with known medicinal properties and an affinity for different cancer cell types can be incorporated when producing AuNP. Our study objective was to develop a minimally invasive technique for the introduction and containment of AuNP within the prostate gland and to document the toxicity and tolerability of these green- synthesized AuNP. Three healthy, adult, male, castrated beagles underwent a minimally invasive surgical procedure under general anesthesia to access the prostate gland. Under fluoroscopic guidance, AuNP were injected into the individual lobes of the prostate (n=3). Each dog was followed closely with clinical monitoring and computed tomography (CT) imaging 1-, 24-, 48-hours and 7 days post-procedure. The procedure was well tolerated, with only transient lethargy noted during the peri-operative period. No consistent findings reflecting systemic toxicity were noted in blood and urine testing. While in the early stages, these results suggest that this novel approach has potential in clinical applications for the treatment of both dog and human patients with prostate tumors.

Funding: OVC Pet Trust

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13) Targeting microRNA to overcome drug resistance in canine lymphoma

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Canine lymphoma is one of the most common malignancies in small animal oncology and bears clinical and pathologic resemblance to non-Hodgkin's lymphoma in humans. In both species, the mainstay of treatment is a multidrug chemotherapy protocol (CHOP) which includes cyclophosphamide (C), doxorubicin (H, hydroxydaunorubicin), vincristine (O, Oncovin), and prednisone (P). Most patients are initially highly responsive to chemotherapy; however, relapsed disease due to the development of drug resistance is common. Furthermore, monitoring of remission relies predominantly on lymph node palpation, and therefore the presence of observable disease. To improve clinical outcomes, there is a substantial need for measurable biomarkers that can detect treatment failure at an earlier stage. microRNA (miRNA or miR) are small RNA molecules that regulate gene expression and have been identified in a number of different tissues in both health and disease. Previous studies in our lab have identified altered miR expression in the tissues and blood of dogs with lymphoma. Moreover, miR have been identified which correlate with response to therapy and the development of drug resistance. The purpose of this study is to determine how miR expression in paired chemosensitive and resistant versions of canine lymphoma and lymphoid leukemia cell lines are altered by exposure to chemotherapy. Real-time reverse transcription-polymerase chain reaction will be used for miR quantification before and after treatment with chemotherapeutic agents. To induce resistance, each cell line will be exposed to stepwise increases in drug concentrations of doxorubicin, vincristine and 4-hydroperoxycyclophosphamide. Lastly, we will attempt to restore chemosensitivity in our resistant cell lines with the use of anti-miRs or miR-mimics, as appropriate based on under- or over-expression in the resistant cells. In addition to identifying miRNA associated with response to therapy, this study will help to identify miRNA involved in resistance to therapy and those with potential to serve as novel therapeutic targets.

Funding: OVC Pet Trust

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14) Evaluation of mitotic count and the impact on survival in dogs with hemangiosarcoma treated with splenectomy and adjuvant doxorubicin or mitoxantrone: 61 cases.

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Introduction: Use of mitotic count (MC) as a prognostic factor has been explored in canine splenic hemangiosarcoma (HSA)[1,2], and was recently reported to be a prognostic indicator in 30 dogs that underwent splenectomy and adjuvant lomustine and doxorubicin[2]. The objective of this retrospective study was to evaluate the impact of MC on survival in a larger cohort of dogs with splenic HSA treated with splenectomy and doxorubicin (n = 60) or mitoxantrone (n = 1). Methods: Retrospective analysis of the medical records of dogs with splenic HSA from 2007 to 2019 at the Ontario Veterinary College was performed. Dogs were included if treated with splenectomy and

adjuvant anthracycline and histopathology reports were available. Log rank test and Cox regression were used to assess the impact of MC on overall survival (OS) and progression free survival (PFS). Results: Sixty-three dogs were included. The median MC of the population was 15. Median PFS and OS were 140 days and 145 days respectively, with a one-year survival rate of 12.7%. Increasing MC was not associated with OS ($p = 0.112$) or PFS ($p = 0.086$). The previously reported cut-off value of 11 as well as the median MC were tested using a Log rank test, though these were not found to be prognostic in the OS of our population ($p = 0.359$ and 0.237, respectively), nor PFS ($p = 0.311$ and 0.212, respectively). Conclusion: A higher MC may not correlate with a shorter OS or PFS in dogs undergoing splenectomy and adjuvant doxorubicin. References: 1. Ogilvie GK, Powers BE, Mallinckrodt CH, Withrow SJ. Surgery and Doxorubicin in Dogs With Hemangiosarcoma. J Vet Intern Med. 1996;10(6):379–84. 2. Moore AS, Rassnick KM, Frimberger AE. Evaluation of clinical and histologic factors associated with survival time in dogs with stage II splenic hemangiosarcoma treated by splenectomy and adjuvant chemotherapy: 30 cases (2011-2014). J Am Vet Med Assoc. 2017;251(5):559 565.

Funding: None to declare.

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15) Semi-quantitative scoring and image analysis protocol for the objective assessment of fluorescence in whole surgical specimens

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Sentinel lymph node (SLN) mapping provides important prognostic information for metastasis. Recently, fluorescence guided surgery is used to identify SLNs and tumor margins with near-infrared (NIR) fluorescence imaging systems. However, these imaging systems lack real-time quantifiable fluorescence analysis. Creation of an analytical workflow consisting of a clinical assessment and post hoc analysis may provide clinical researchers a method to quantify intraoperative fluorescence to improve objective outcome measures in clinical trials. A scoring system and validated image analysis are used to determine the amount and intensity of fluorescence within surgical specimens *in vivo* and *ex vivo*. Canine lymph nodes were extirpated during lymph node mapping using a NIR fluorescent dye called indo-cyanine green (ICG). A semi-quantitative assessment was used to score the amount of fluorescence on lymph nodes. Standardized imaging with a NIR exoscope was used for image analysis to determine ICG fluorescence thresholds and measure fluorescence amount and intensity. Post hoc fluorescence quantification (threshold of Hue = 165-180, Intensity = 30-255) demonstrated strong agreement with semi-quantitative scoring ($k = 0.9734$, $p < 0.0001$). Fluorescence intensity with either threshold of 35-255 or 45-255 were significant predictors of fluorescence and had high sensitivity and specificity ($p<0.05$). Fluorescence intensity and quantification had a strong association ($p<0.001$). Validating a semi-quantitative scoring system by image analysis allows for an objective evaluation of fluorescence in situ. The rigorous thresholding for ICG intensity provides stringent post hoc quantification of fluorescence when imaging systems lack quantification algorithms.

Funding Source: OVC Pet Trust

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16) ICCI comparative oncology program: Utilizing spontaneous companion animal cancers in clinical research studies as models for human cancers

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Similar to people, cancer is common in companion animals (CA) with ~1:3 dogs and 1:7 cats developing cancer and ~50% of pets >10 years old dying of the disease. Many CA cancers share similar characteristics and disrupted pathways to human cancer types. Hence studies in CA cancer patients enable valuable clinical data to be obtained for translational research relevant to human cancer as well as benefiting veterinary patients, e.g. novel techniques and treatment options. Oncology-related clinical research trials at OVC HSC are performed with the Institute for Comparative Cancer Investigation (ICCI). The ICCI can assist with the administrative aspects of OVC HSC-based CA clinical research projects, including required paperwork, recruiting patients, obtaining informed client consent, facilitating sample collection, liaising with referring veterinarians and publicity. Furthermore, the ICCI was the first Canadian member in the National Institute of Health-National Cancer Institute (NIH-NCI) Comparative Oncology Trials Consortium (COTC), which conducts international multi-centred studies. Since 2014, over 1320 patients have been recruited into 45 oncology-related studies (many OVC Pet Trust funded) at the OVC HSC. Currently there are 10 studies recruiting oncology patients: 8 canine, 1 feline and 1 both species (http://ovc.uoguelph.ca/icci/trials) and another 4 studies are closed for recruitment but are still actively monitoring and collecting samples from recruited patients. Three of the closed studies are COTC collaborations, investigating osteosarcoma in dogs which is of particular relevance to human pediatric osteosarcoma. Therefore, the ICCI comparative oncology program has the potential to facilitate improving the healthcare and lives of companion animals and also of people.

Funding: OVC Pet Trust, Smiling Blue Skies Cancer Fund, Morris Animal Foundation

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17) Expression and function of the EphB4 receptor in canine and human osteosarcoma

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Osteosarcoma is the most common bone cancer in canines and humans. In both species, osteosarcoma is highly aggressive with a high rate of metastasis to the lungs. Current treatment involves chemotherapy and either a limb-sparing surgery for humans or limb amputation for dogs. Advances in treatment options for osteosarcoma has been limited and there is a need for the development of more effective therapeutic approaches. Erythropoietin-producing hepatocellular (Eph) receptors are the largest group of receptor tyrosine kinases with nine EphA and five EphB members. The Eph receptors regulate many cellular activities including differentiation, movement, adhesion, proliferation, and survival. Effects of altered expression or mutation of Eph receptors on tumor aggressiveness have been characterized in multiple human malignancies, making these receptors attractive targets for therapeutic intervention. In human and canine osteosarcoma however, the expression and function of the EphB receptors has been poorly characterized. Due to the high degree of physiological and cellular similarity between canine and human osteosarcoma,

we employ a comparative approach to investigate the role EphB receptors perform in promoting osteosarcoma. In this project, we initially performed a comprehensive expression analysis of all EphB receptors in multiple dog osteosarcoma cell lines. Utilizing both traditional real-time and state-of-the-art droplet digital PCR, we found the upregulated expression of EphB4 in canine osteosarcoma when compared to control canine osteoblasts, a result confirmed by assessment of these proteins by Western blotting. The expression of EphB4 was further investigated in human osteosarcoma cells. Silencing EphB4 expression reduced cell proliferation in both canine and human osteosarcoma. Future studies will elucidate the effect upregulated EphB4 receptor expression has on additional cell functions *in vitro* including cell survival, migration, and invasion. The *in vivo* effect of EphB4 function in osteosarcoma will also be assessed by monitoring tumor development and invasiveness when EphB4 expression is silenced in mouse xenograft models.

Funding: Allard Research Chair Start-up Fund, Natural Sciences and Engineering Research Council of Canada (NSERC) Canada Graduate Scholarship – Master's

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18) The ICCI Companion Animal Tumour Sample Bank: facilitating translational cancer research

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The Companion Animal Tumour Sample Bank (CATSB) continues to successfully facilitate basic and translational veterinary oncology research. Currently, CATSB has over 1500 cases banked and has contributed samples to 23 intramural and extramural research projects. Located in the OVC HSC Mona Campbell Centre for Animal Cancer, the CATSB is the only veterinary oncology tissue bank in Canada and is registered with the Canadian Tissue Repository Network. Sample types collected and stored at ultra-cold temperature are serum, plasma, buffy coat, urine, and tissue. Tissue samples (tumour and matched normal) are collected immediately following surgical excision and are available as flash frozen, in RNAlater, and in CryoMatrix. Tumour tissue is also formalin fixed, paraffin embedded, sectioned, and H&E stained for quality control analysis by a pathologist. Prospective sampling can also be tailored to suit the specific needs of researchers. The three most prevalent canine tumour types in the CATSB are soft tissue sarcoma, lymphoma, and osteosarcoma, but a variety of other neoplasms has also been banked. There are also currently 11 primary cell lines from canine and feline tumours available that have been characterized at various levels, with more in development. In addition to samples, researchers can receive patient signalment, histopathology, and follow-up clinical data. Researchers access samples by filling out a short application form. A cost-recovery fee (which is subsidized for University of Guelph researchers) is applied to enable the CATSB to continue its mission: to facilitate veterinary research to improve the lives of companion animals with cancer, with the potential to contribute to comparative human cancer research.

Funding: OVC Pet Trust, Smiling Blue Skies Cancer Fund

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19) DNA methylation levels and epigenetic enzyme expression for prognostication of canine mast cell cancer.

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Canine mast cell tumors (MCTs) constitute up to 21% of all canine skin tumors. In Patnaik grade 2 and Kiupel low grade MCTs, biological aggressiveness is sometimes difficult to predict. DNA hypermethylation, hypomethylation and epigenetic enzyme dysregulation are involved in the progression of various cancers. Therefore, global levels of DNA 5-methylcytosine, 5 hydroxymethylcytosine and DNA methylation enzyme expression could serve as prognostic biomarkers to predict MCT aggressiveness and better guide therapies. To quantify the levels of global DNA methylation, hydroxymethylation, and enzymes involved with these DNA modifications and their relationship with canine MCT outcome a tissue microarray (TMA) was used. A TMA consisting of cores from 244 different tumor samples from 189 dogs, along with associated outcome data was immunolabelled for 5-methylcytosine (5MC), 5 hydroxymethylcytosine (5hMC) as well as members of the DNA methyltransferase (DNMT), Ten-eleven Translocation (TET) and Isocitrate dehydrogenase (IDH) enzyme families. H-scores were generated for the cores using QuPath (v0.1.2) software. Patnaik grade 3 MCTs had significantly higher DNMT3a H-scores versus grade 1 and 2 MCTs. Kiupel Low grade and Patnaik grade 2 MCTs with high 5MC H-scores also had poorer overall survival (OS). When not using grade to stratify cases, MCTs with 5M Hscores had poorer disease-free interval (DFI) in all cases and in subcutaneous only cases. MCTs with high 5MC H-scores had shorter OS in all, dermal, and subcutaneous MCT cases. When looking at MCTs in the Kiupel low grade group, Patnaik grade 2 group, and cases with no adjuvant therapy, cases with high 5MC H-scores had shorter OS compared to cases with low H-scores. High DNMT3a H-scores had poorer DFI and OS for all cases and shorter OS for dermal cases. For MCTs with no adjuvant therapy, high DNMT3a H-scores had shorter OS compared to low H-scores. To further investigate the relationship between epigenetics and canine MCTs, analysis of DNMT1, IDH and TET enzyme expression in this TMA is ongoing. The impact of 5-Aza-2′-deoxycytidine on cultured canine MCT cell survival and enzyme expression is also under evaluation. Based on the results to date 5-methylcytosine and DNMT3a have potential as prognostic biomarkers in canine MCTs, especially for intermediate histopathological grade.

Funding Source: OVC Pet Trust, OVC Graduate Stipend Program.

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20) *In vitro* analysis of oxidative stress on off-target infection of activated T cells by oncolytic vesicular stomatitis virus

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The rapidly progressing field of oncolytic virotherapy takes advantage of the ability of genetically modified viruses to selectively infect and lyse tumour cells. Oncolytic viruses (OVs), such as vesicular stomatitis virus (VSV), preferentially target cancerous cells due to various mechanisms

ranging from exploitation of cancer-specific receptors and utilization of the defective antiviral defenses of tumour cells. However, success has been limited, partially due to the ability of some OVs, like VSV, to cause off-target infection of leukocytes including activated CD4+ and CD8+ T cells, thus reducing its therapeutic potential. Previous studies have demonstrated that antioxidants can prevent viral infection and replication due to their ability to reduce concentrations of reactive oxygen species (ROS). The goal of this study was to determine whether certain antioxidants could prevent infection of activated T cells by VSV. Splenocytes from female C57BL/6 mice were exposed to antioxidants *in vitro* and flow cytometry was used to ascertain the percentage of activated T cells infected with VSV after 18 hours. Treatment with N-acetyl-Lcysteine (NAC) or catalase decreased the frequency of infection with VSV. Additionally, a 2',7'–dichlorofluorescin assay used to quantify ROS demonstrated a significant decrease oxidative stress in cells treated with catalase. The low-density lipoprotein receptor is the primary protein used by VSV to gain entry into cells. Its expression, as measured by mean fluorescence intensity, on T cells was quantified by flow cytometry and was found to decrease on cells treated with NAC. These results have the potential to improve multi-dosing cancer immunotherapies by decreasing off-target infection of effector T cells when paired with antioxidants already approved for clinical use.

Funding: Program Project Grant (Terry Fox Research Institute), Operating Grant (jointly funded by Canadian Institutes of Health Research - Institute of Cancer Research and the Cancer Research Society), Vanier Canada Graduate Scholarship, Brock Doctoral Scholarship, and the Ethel Rose Charney Scholarship in the Human/Animal Bond.